COBOP: Microbial Biofilms: A Parameter Altering The Apparent Optical Properties Of Sediments, Seagrasses And Surfaces.

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LONG-TERM GOAL

The long-term goal of my research is to understand the optical properties of microbial biofilms, which form coatings on sediments and other surfaces in coastal oceans. The specific project goals are to determine how biofilm coatings may influence (i.e., alter) optical spectra of sediments and other surfaces through reflection, scattering and fluorescence. This project is a part of the CoBOP (Coastal Ocean Benthic Optical Properties) initiative in the Environmental Optics Program.

OBJECTIVES

The objective of year three was to perform the second component of our field-study" at Lee Stocking Island in association with the CoBOP "Sediment Group, and to begin interactive studies with CoBOP Optics investigators. Our specific objectives were to:
1) extract exopolymers from several sediment field sites having different microbial biofilm characteristics (as determined from results from year two); 2) Characterize absorbance properties of exopolymers in gel and solution states; 3) conduct near-field sediment/exopolymer manipulative experiments to determine how spectral reflectance may be altered by the presence of exopolymers (S-2000 Spectral Reflectance); 4) Conduct initial far-field field sediment/exopolymer experiments which manipulate the exopolymer concentrations in sediments, and determine effects on reflectance measurements (using fluorescence induced laser line scan (FILLS). 5) Profile natural biofilms in sediments using confocal laser microscopy to determine how biofilm coatings on sediments may alter the spacing of sediment grains, and hence alter reflectance properties of sediments.

APPROACH

Three sediment sites were intensively sampled to isolate natural biofilm exopolymers from sediments, and to characterize the intact biofilms that coat sediments using confocal laser microscopy (CLM). Absorbances, spectral reflectances, and fluorescence properties of exopolymers were determined. Manipulative experiments were conducted in which the exopolymer concentrations of sediments were varied. Then spectral reflectance measurements were determined using S-2000 spectrometers (in the lab), and compared with similar manipulations in the field using fluorescence-induced laser line scan (FILLS) in collaboration with Dr. Mike Strand and colleagues. Finally, the effects of biofilms on the quenching of fluorescence was examined by experimentally growing biofilms on fluorescent surfaces, which mimic mine-like objects (MLOs). Fluorometric measurements were conducted over a wide spectral range of excitations and emissions to determine the nature of the quenching effects. Follow-up

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laboratory experiments were conducted to determine the relative roles of quenching effects resulting from biofilm cell-pigments and from biofilm polymers.

WORK COMPLETED

Our field campaign to Lee Stocking Island during year-three was highly successful. All objectives were accomplished. Three different sediment sites were intensively examined, owing to their observed differences in biofilm properties. Measurements of Spectral Reflectance, and fluorometric analyses were conducted as outlined above. Quantitative imaging, generated by nanoplast-embedded natural sediments, were generated by scanning confocal laser microscopy.

RESULTS

Results show that certain sediment sites, which contain an abundance of biofilm mat material, include the "Grapestone site", Twin Beaches (diatom mat) sites, and the North Perry (diatom mat) sites. Sediments which contained a high abundance of biofilm material (i.e. exopolymers) showed a greater relative spacing of sediment grains (Fig.1) due to the presence of exopolymer. Biofilms, such as a diatom mat caused a reduced overall spectral reflectance (Fig. 2). The overall reductions in reflectance are estimated at approx. 20%. This results from the exopolymer gel fraction (not the cells) of the biofilm. Since the exopolymers do not exert specific absorbances (Fig. 3), the observed reductions may be due to enhance scattering by the exopolymer. The presence of exopolymer gels of biofilms alter the relative spacing of sediment grains (Fig. 1) when biofilm mats are present. A second major effect of biofilms is that the fluorescence signatures of an underlying surface may be "quenched" by the presence of a biofilm community of just 20 to 40 um thickness on that surface (Fig. 4). We are currently refining our experimental manipulations to determine the exact mechanisms of these changes in sediment optical properties resulting from the presence of Biofilms and their Exopolymers.

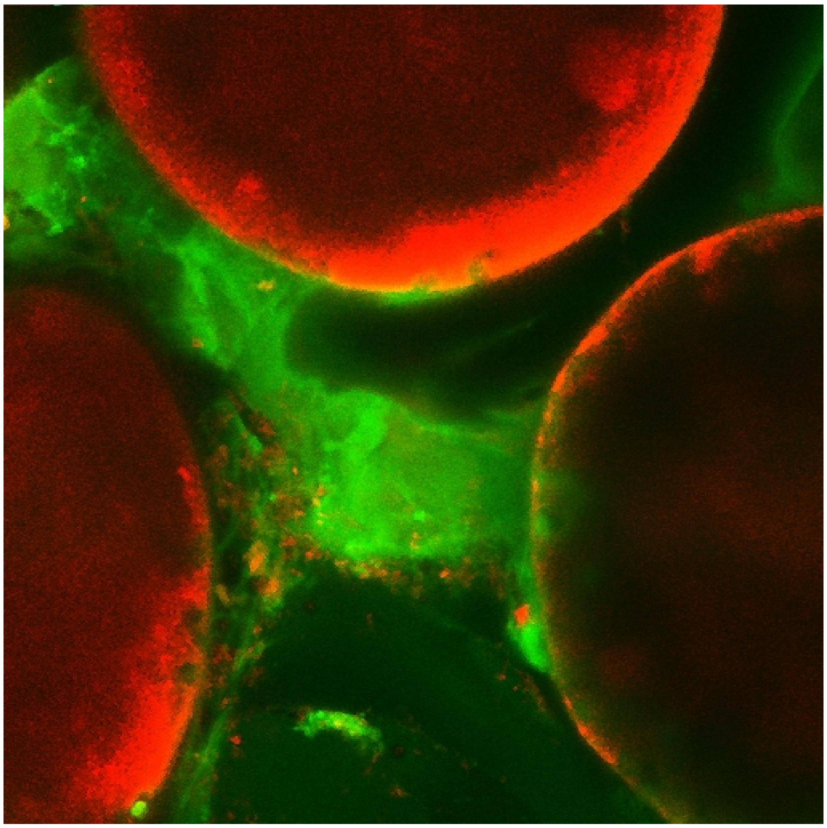

Fig.1. A scanning confocal laser micrograph showing sediment containing biofilm exopolymers (GREEN). The sediment particles or ooids (RED) are separated by the exopolymer gel material of the biofilm. This may significantly alter the optical properties of sediments by changing the relative spacing of sediment grains when biofilms are present.

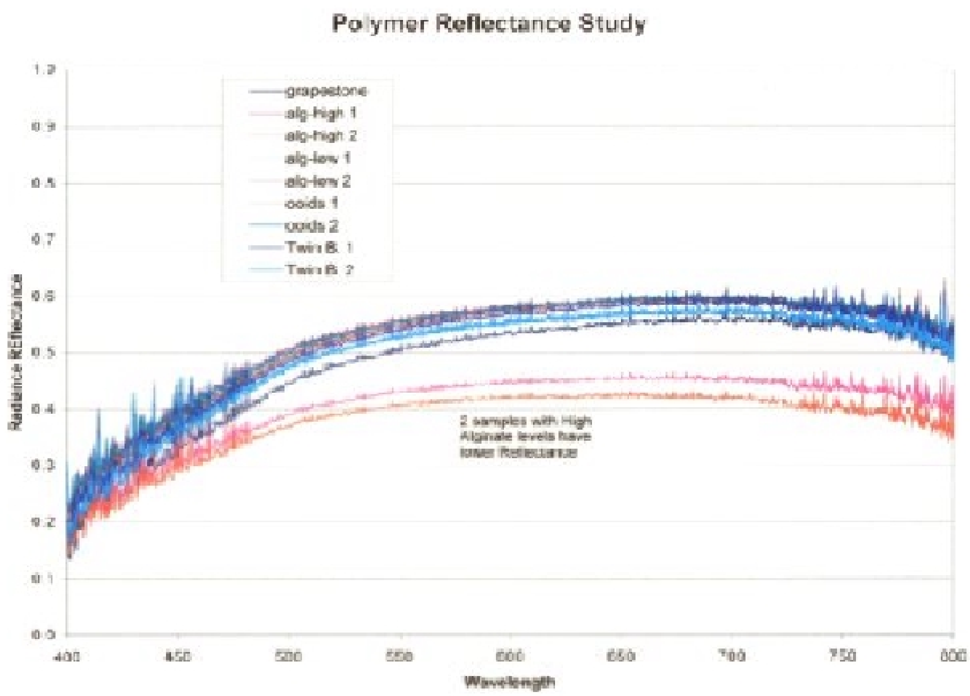

Fig. 2. Graph showing Spectral Reflectance of Sediments having high concentrations of Exopolymer-Gel coatings (RED & ORANGE), and sediments having low concentrations (BLUE). The presence of exopolymer coatings on sediments greatly reduces the spectral reflectance properties.

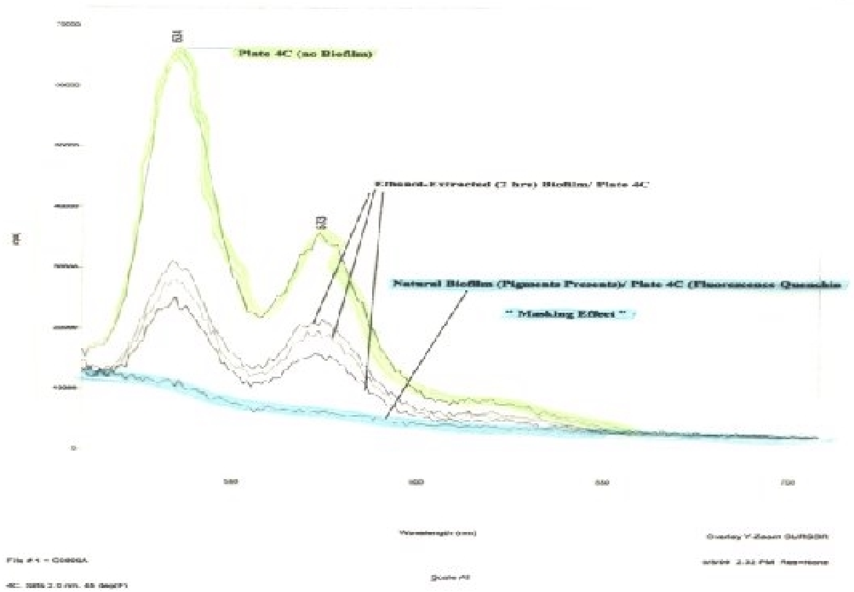

Fig. 3. Fluorescence spectral graph showing the Quenching of an underlying fluorescent surface due to the presence of a microbial biofilm on that surface. The surface (without a biofilm) is shown in YELLOW. When a cyanobacteria biofilm is grown on the surface (BLUE), the fluorescence signature is significantly reduced. The presence of a microbial biofilm exerts a "masking effect" on the fluorescence signature of the underlying surface.

IMPACT/APPLICATION

Our previous results have shown that biofilms occur in varying amounts, and at virtually all sediment sites. At sites where biofilms occur in high or patchy abundance, they may exert significant alterations on the optical spectra of sediment. These alterations are detectable using a range of instrumentation. Our developing studies suggest that biofilms may potentially affect the specific optical signatures of surfaces such as MLO (mine like objects). This may occur through their reductions in reflectance and quenching of fluorescence. Therefore, "microscopic" microbial biofilms coatings on sediments, which are not readily obvious, may exert strong effects on optical signatures of sediments.

TRANSITIONS

The close coordination of “Sediment group” CoBOP personnel has provided a strong and unique dimension to our work. We are conducting co-ordinated field experiments with other members of the Sediment Group in multi-investigator field experiments.

A second coordination involves work in conjunction with Dr. Mike Strand using the Laser Line Scan System. This work involves manipulative experiments of sediment systems and subsequent quantitative imaging using the laser line scan. The purpose of this work is to quantitatively determine the effects of sediment-associated biofilms on alterations in Sediment Reflectance and Fluorescence profiles.

RELATED PROJECTS

None

REFERENCES

None

PUBLICATIONS

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